The Definition of ABO Factors in Transplantation: Relation to Other Humoral Antibody States

T.E. Starzl, A. Tzakis, L. Makowka, B. Banner, A. Demetrius, G. Ramsey, R. Duquesnoy, and M. Griffin

THE GUIDELINES for solid organ transplantation from donors to recipients of different blood types were established by observations in a relatively small number of renal transplantations carried out in 1962 and 1963.1-4 In this presentation, we will retrace these steps, suggest how lessons with ABO mismatches are applicable to other preformed antibody states (including those of xenotransplantation), and show how modern day strategies with antibody depletion and pharmacologic manipulation of mediators of the inflammatory response could be exploited therapeutically to prevent hyperacute rejection.

HYPERACUTE REJECTION
Kidney Transplantation

The first experience was reassuring. On January 31, 1963, a 38-year-old man of A+ blood type was given a kidney from his sister whose blood type was B+. Despite a moderately severe rejection at 25 days, the kidney survived and still functions perfectly more than 24 years later. On March 19 and July 29 of the same year, two A to O transplantations were performed, one from a cadaveric donor and the other from a mother, also without perioperative incident.

It looked as if ABO matching was irrelevant in renal transplantation and a manuscript with this conclusion was accepted by Surgery.1 While the article was still in press, its message was changed drastically by two catastrophic experiences, one with an A to O transplantation and the other with a B to O transfer. Both kidneys were hyperacutely rejected within minutes.

Arteriograms of the removed specimen showed nonfilling of the small vessels and this correlated histopathologically with widespread thrombotic occlusion of the microvasculature. It was concluded that the immediate rejection had been precipitated by high affinity isoagglutinins in the recipient sera, which had bound with A or B antigens in the mismatched kidneys. The logic of the conclusion was supported by the documentation 2 or 3 years earlier by Szulman5 and by Hogman that the ABO antigens were widely distributed throughout human tissues and organs.

An addendum was added to the Surgery article including the now familiar table showing permissible patterns of tissue transfer that were designed to avoid placing kidneys into an environment containing antigraft isoagglutinins (Table 1). Data on isoagglutinin titers was subsequently provided.6 Later, in a classical report, Rapaport et al7 showed how sensitization of human volunteers with purified A or B antigens caused increased titers of isoagglutinins and accelerated (white graft) rejection of subsequently transplanted skin grafts. The circle of evidence seemingly was complete. However, it is worth emphasizing that not all of the mismatched kidneys had rejected and that those that escaped immediate destruction did not seem to pay a later penalty.

Much of the recent interest in the ABO system by transplanters has been focused on
reliably surmounting the acute antibody barrier, thereby expanding the available pool of organs. The recent use of A₂ kidneys for O recipients is an example. The practice is based on the reports by Breimer and Brynger et al. of Sweden who showed that the A antigen is poorly represented in the kidneys of nonsecretor individuals or in patients with A₂ blood type. The assumption has been that kidneys from such donors would not be the target of the anti-A isoagglutinins in O or B recipients.

However, this newest attempt to ride over an ABO barrier may not be completely safe. On December 28, 1986 in Pittsburgh, a 39-year-old male of O blood type was given a kidney from an A₂ cadaveric donor. Cold ischemia time was 35½ hours. The kidney underwent hyperacute rejection within five minutes. The anti-A, titers of both IgG and IgM were high by comparison with those in other candidates for kidneys, livers, or hearts (Table 3).

Histopathologic examinations showed the same lesions as in the hyperacutely rejected ABO incompatible kidney 25 years previously (Fig 1). IgM and complement were found in the vessel walls (Fig 1). Fortunately, an O kidney became available while the wound was still open and it was inserted with a perfect result.

**Extrarenal Organs**

How much relevance the guidelines summarized in Table 1 will have to do with other organs is still being determined. For many years, it has been appreciated that the liver is resistant to hyperacute rejection. Today reports will be given from three different centers about what the risks and probabilities actually are in using ABO incompatible livers.

There have been a few accidental heart transplantations across ABO barriers. In Pittsburgh, there have been two such cases. One recipient has a perfect result after 18 months, but the other died in less than a day with accelerated rejection (Table 4).

To leave this underdeveloped subject, our opinion is that there is a small but real jeopardy in crossing these forbidden ABO barriers in heart and liver recipients unless some specific provisions such as depletion of isoagglutinins or pharmacologic therapy is carried out as we will mention later.

**HUMORAL ANTIBODY STATES**

<table>
<thead>
<tr>
<th>Table 1. Direction of Acceptable Mismatched Tissue Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>O to non-O</td>
</tr>
<tr>
<td>Rh⁻ to Rh⁻</td>
</tr>
<tr>
<td>Rh⁻ to Rh⁺</td>
</tr>
<tr>
<td>A to non-A</td>
</tr>
<tr>
<td>B to non-B</td>
</tr>
<tr>
<td>AB to non-AB</td>
</tr>
</tbody>
</table>

**Table 2. Isoagglutinin Titers Before and After Hyperacute Rejection of a Kidney From an A₂ Nonsecretor Donor to an O Recipient**

<table>
<thead>
<tr>
<th>Anti-</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>IgM</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>&gt;512</td>
</tr>
<tr>
<td>A₂</td>
<td>IgM</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>1,024</td>
</tr>
<tr>
<td>B</td>
<td>IgM</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>512</td>
</tr>
</tbody>
</table>

NOTE. O is universal donor; AB is universal recipient.

**Table 3. Isohemagglutinin Titers in Group O Liver, Heart, or Renal Transplant Candidates**

<table>
<thead>
<tr>
<th>Anti-A₁ (n = 53)</th>
<th>Anti-A₂ (n = 24)</th>
<th>Anti-B (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A₁ IgM</td>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td>Antii-A₂ IgM</td>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td>Anti-B IgM</td>
<td>IgG</td>
<td>IgG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Median</th>
<th>128</th>
<th>256</th>
<th>32</th>
<th>64</th>
<th>128</th>
<th>256</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected range*</td>
<td>32-256</td>
<td>64-1,024</td>
<td>≤16-256</td>
<td>≤16-512</td>
<td>≤16-256</td>
<td>≤16-1,024</td>
</tr>
</tbody>
</table>

NOTE. IgM, 60-minute room temperature saline incubation; IgG, 60-minute 37°C saline incubation, then anti-IgG antoglobulin.

*90% of patients are in these ranges.
Fig 1: (A) Glomerulus with congested capillary loops, which contain platelet-fibrin thrombi and many neutrophils (hematoxylin-eosin, original magnification x 400). (B) Immunofluorescent stain for anti-IgM (original magnification x 400) with dense deposition of IgM in polar arteriole.
Table 4. Heart Transplants Across ABO Barrier (Pittsburgh)

<table>
<thead>
<tr>
<th>Case</th>
<th>ABO</th>
<th>Recipient</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AB</td>
<td>A</td>
<td>Good, 18 mo</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>O</td>
<td>Died of rejection 12 h</td>
</tr>
</tbody>
</table>

**HUMORAL ANTIBODY STATES**

**HYPERACUTE REJECTION WITH NON-ABO ANTIBODIES**

It was not long after the description of hyperacute renal rejection with ABO mismatches that a similar phenomenon was described by Terasaki in a patient with anti-graft preformed lymphocytotoxic antibodies. The concept that immediate graft rejection could be caused by preformed antibodies was confirmed and extended by Kissmeyer Nielsen, Patel and Terasaki, Williams et al, and many others. Other systems, such as the anti-vascular endothelial cell antibodies discussed by Cerilli elsewhere in this symposium, appears to precipitate the same kind of hyperacute rejection of various organs. Thus, the initiating antibodies or other circumstances have differed, but a final common pathway of destruction has been involved in all.

**THE EFFECTOR CASCADE**

About 20 years ago, we made serious attempts to delineate that common pathway in a series of inquiries in patients and animals that were strongly influenced by collaborations with the experimental pathologist, Frank J. Dixon of La Jolla, CA and the immunologist, Paul I. Terasaki. Two experimental models were used. One was the dog that was hypersensitized with multiple surface or buried skin grafts. Kidney, liver, or spleen transplantation from the sensitizing donor was then performed. The other experimental model was the pig to dog xenograft. The dog had preformed hetero-specific cytotoxic antibodies that led to hyperacute rejection of the pig kidney within 5 to 30 minutes. In addition, an opportunity presented to observe the events of hyperacute rejection in a small number of hypersensitized human kidney recipients. From these observations the hypothesis was developed that the initiating step of hyperacute rejection was antibody induced, but that the irreversible injury patterns resulted from a cascade of secondary and nonspecific events. It was shown that antibodies, clotting factors, and formed blood elements were rapidly cleared by the grafts as estimated by gradient measurements.

Fibrinolysis from the renal vein also was a consistent finding, and in extreme cases, a disseminated intravascular coagulation (DIC) could be produced. Various descriptive terms have been applied to hyperacute rejection and its different aspects have been emphasized by comparison to the Arthus reaction, inverse anaphylaxis, and the generalized Swartzman reaction. Each of these classic immunologic phenomena is, or can be, initiated by interaction of antibody and antigen, and in each there are multiple secondary events including inflammation and coagulation. It was our conclusion that hyperacute rejection was "an immunologically mediated coagulopathy that led to the devascularization and destruction of a transplanted organ, and which sometimes resulted in systemic coagulation abnormalities."

At the time this work was going on, there was little detailed information about the mediators of the inflammatory response. The modern understanding of the inflammatory response following a variety of initiating events including antigen-antibody reactions is summarized by Makowka elsewhere in this symposium.

**THERAPEUTIC INTERVENTION FOR HYPERACUTE REJECTION**

**Antibody Removal**

Almost two decades ago, therapeutic efforts to treat preformed antibody states were made in spite of the above described lack...
of knowledge. The most obvious approach was to eliminate the offending antibodies. The first attempt to our knowledge was made by Merkel and Bier et al.\textsuperscript{19,22} Using a primitive plasmapheresis machine, it was possible to extend many times the life of the pig to dog renal xenografts. A number of clinical trials of plasmapheresis to reduce antibodies have been reported through the years but with unpredictable and often uninterpretable results. Plasmapheresis has been used by the group at the Catholic University of Louvain for reduction of isoagglutinin titers before transplantation across ABO barriers (summary report elsewhere in this volume).

Thoracic duct drainage is an alternative technique to reduce antibodies. Even though thoracic duct drainage removes T-lymphocytes, which are not known to effect antibody function, there is an accompanying drastic reduction by unexplained mechanisms of all of the immunoglobulin classes.\textsuperscript{23,24} Machleder and Paulus\textsuperscript{23} showed that isoagglutinins could be virtually eliminated with 3 or 4 weeks of thoracic duct drainage.

Antibody reduction by any currently available techniques is of limited value and duration, but technologic developments with the so-called staphylococcal protein A adsorbent columns\textsuperscript{25} will make it possible within the next year or so to virtually remove specific classes of immunoglobulins for long periods of time perioperatively.

\textit{Altering the Effector Cascade}

When it was first appreciated that the effector cascade of hyperacute rejection was a relatively nonspecific one involving coagulation, the simple expedient of using heparin for anticoagulation was tried, but with no benefit.\textsuperscript{26} Other means to prevent coagulation or complement activation have included the use of snake venom in experimental animals. Research of this kind came to an abrupt standstill almost 15 years ago because of the great difficulty in achieving anything of practical therapeutic value. The most successful technique with either the difficult pig to dog model or after sensitization with skin homografts was the intraarterial infusion of citrate.\textsuperscript{27} Citrate in toxic doses can be a profound anticoagulant but it has so many other biologic effects including participation in complement activation that the specificity of action could not be clarified.

With the development of new drugs with which one can intervene at specific levels of the inflammatory response, the prospect of mitigating or preventing hyperacute rejection has become more practical, as will be discussed by Leonard Makowka later this morning. The work that Dr Makowka will report should be directly applicable to the ABO questions central to this meeting.

\textbf{GRAFT-VERSUS-HOST CONSIDERATIONS}

So far, we have discussed only how preformed recipient antibodies can damage ABO mismatched grafts. It has become well recognized that grafts containing lymphoid tissue can act as B cell organs and elaborate isoagglutinins or other antibodies that can bind with host tissues. In one of our A recipients who was given an O pancreaticoduodenal-splenic graft, the spleen produced such high titers of anti A isoagglutinins that a hemolytic crisis was caused.\textsuperscript{27} Livers predictably cause hemolysis under similar circumstances\textsuperscript{28} but less severely. Other syndromes such as thrombocytopenia may result. The possibility of this kind of humoral graft-versus-host reaction exists even with the kidney.\textsuperscript{29} Such observations have made it less and less desirable to perform transplantation from universal donors and with other so-called compatible but not identical combinations, especially with nonrenal organs.

\textbf{SUMMARY}

The first examples of hyperacute rejection of renal homografts were seen almost 25 years ago when kidneys were transplanted to ABO incompatible recipients whose plasma contained antigraft isoagglutinins. Hyperacute
rejection caused in sensitized recipients by lymphocytotoxic antibodies is similar in that the immune reaction triggers an acute inflammatory reaction that leads to widespread thrombotic occlusion and devascularization of the graft. The events after xenotransplantation between certain species are essentially the same. Potential strategies to avoid the precipitating antigen antibody reaction or to mitigate the resulting effector cascade are described.

REFERENCES

5. Hogman CF: Vox Sang 4:12, 1959